

CLAIMS

What is claimed is:

1. An isolated nucleic acid sequence encoding a polypeptide with isoflavone synthase activity having the amino acid sequence set forth in SEQ ID NO:66 wherein

- 5 Xaa₁₀ is Phe or Leu
 Xaa₁₆ is Ser or Leu
 Xaa₂₃ is Ser or Thr
 Xaa₂₅ is Ile or Lys
 Xaa₃₉ is Lys or Arg
10 Xaa₄₈ is Pro or Leu
 Xaa₆₀ is Pro or Leu
 Xaa₇₃ is Leu or His
 Xaa₇₄ is Ser or Tyr
 Xaa₉₅ is Ala or Thr
15 Xaa₉₆ is Asn or His
 Xaa₁₀₂ is Asn or Ser
 Xaa₁₁₀ is Ile, Val, or Thr
 Xaa₁₁₂ is Arg or His
 Xaa₁₁₇ is Asn or Ser
20 Xaa₁₁₈ is Ser or Leu
 Xaa₁₂₁ is Met or Arg
 Xaa₁₂₂ is Ala or Val
 Xaa₁₂₄ is Phe or Ile
 Xaa₁₂₉ is Lys or Arg
25 Xaa₁₄₇ is Lys or Glu
 Xaa₁₅₉ is Leu or Phe
 Xaa₁₆₂ is Ala or Val
 Xaa₁₆₆ is Ser or Gly
 Xaa₁₇₀ is Gln or Arg
30 Xaa₁₇₅ is Val or Leu
 Xaa₁₈₃ is Ala or Thr
 Xaa₁₈₇ is Thr or Ile
 Xaa₁₉₁ is Met or Val
 Xaa₂₀₉ is Phe or Tyr
35 Xaa₂₁₉ is Arg or Trp
 Xaa₂₂₃ is Tyr or His
 Xaa₂₅₃ is Gly or Glu
 Xaa₂₅₉ is Lys or Glu

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5 Xaa₂₆₃ is Val or Asp
 Xaa₂₆₄ is Val, Asp, or Ile
 Xaa₂₆₈ is Ala or Val
 Xaa₂₇₂ is Phe or Leu
 Xaa₂₈₅ is Thr or Met
 Xaa₂₉₃ is Glu or Asp
 Xaa₂₉₄ is Thr, or Ile
 Xaa₃₀₁ is Phe or Leu
 Xaa₃₀₆ is Thr or Ile
 10 Xaa₃₁₁ is Val or Glu
 Xaa₃₁₂ is Val or Ala
 Xaa₃₂₅ is Arg or Lys
 Xaa₃₂₈ is Gln or Glu
 Xaa₃₃₄ is Val or Ala
 15 Xaa₃₄₂ is Arg or Ile
 Xaa₃₇₇ is Thr or Ile
 Xaa₃₈₁ is Glu or Gly
 Xaa₃₈₅ is Tyr, His, or Cys
 Xaa₃₈₇ is Ile or Thr
 20 Xaa₃₉₃ is Val or Ile
 Xaa₃₉₄ is Leu or Pro
 Xaa₄₀₂ is Arg or Lys
 Xaa₄₀₄ is Ser or Pro
 Xaa₄₁₃ is Ser or Phe
 25 Xaa₄₂₂ is Glu or Gly
 Xaa₄₂₈ is Gly or Arg
 Xaa₄₂₉ is Pro or Leu
 Xaa₄₃₅ is Gln or Arg
 Xaa₄₄₇ is Arg or Gly
 30 Xaa₄₅₃ is Asn, Ser, or Ile
 Xaa₄₅₉ is Met or Thr, and
 Xaa₄₈₅ is Asp or Gly.

2. An isolated polypeptide sequence of SEQ ID NO: 66 wherein

35 Xaa₁₀ is Phe or Leu
 Xaa₁₆ is Ser or Leu
 Xaa₂₃ is Ser or Thr
 Xaa₂₅ is Ile or Lys
 Xaa₃₉ is Lys or Arg

Xaa₄₈ is Pro or Leu
Xaa₆₀ is Pro or Leu
Xaa₇₃ is Leu or His
Xaa₇₄ is Ser or Tyr
Xaa₉₅ is Ala or Thr
Xaa₉₆ is Asn or His
Xaa₁₀₂ is Asn or Ser
Xaa₁₁₀ is Ile, Val, or Thr
Xaa₁₁₂ is Arg or His
Xaa₁₁₇ is Asn or Ser
Xaa₁₁₈ is Ser or leu
Xaa₁₂₁ is Met or Arg
Xaa₁₂₂ is Ala or Val
Xaa₁₂₄ is Phe or Ile
Xaa₁₂₉ is Lys or Arg
Xaa₁₄₇ is Lys or Glu
Xaa₁₅₉ is Leu or Phe
Xaa₁₆₂ is Ala or Val
Xaa₁₆₆ is Ser or Gly
Xaa₁₇₀ is Gln or Arg
Xaa₁₇₅ is Val or Leu
Xaa₁₈₃ is Ala or Thr
Xaa₁₈₇ is Thr or Ile
Xaa₁₉₁ is Met or Val
Xaa₂₀₉ is Phe or Tyr
Xaa₂₁₉ is Arg or Trp
Xaa₂₂₃ is Tyr or His
Xaa₂₅₃ is Gly or Glu
Xaa₂₅₉ is Lys or Glu
Xaa₂₆₃ is Val or Asp
Xaa₂₆₄ is Val, Asp, or Ile
Xaa₂₆₈ is Ala or Val
Xaa₂₇₂ is Phe or Leu
Xaa₂₈₅ is Thr or Met
Xaa₂₉₃ is Glu or Asp
Xaa₂₉₄ is Thr, or Ile
Xaa₃₀₁ is Phe or Leu
Xaa₃₀₆ is Thr or Ile

Xaa₃₁₁ is Val or Glu
 Xaa₃₁₂ is Val or Ala
 Xaa₃₂₅ is Arg or Lys
 Xaa₃₂₈ is Gln or Glu
 Xaa₃₃₄ is Val or Ala
 Xaa₃₄₂ is Arg or Ile
 Xaa₃₇₇ is Thr or Ile
 Xaa₃₈₁ is Glu or Gly
 Xaa₃₈₅ is Tyr, His, or Cys
 Xaa₃₈₇ is Ile or Thr
 Xaa₃₉₃ is Val or Ile
 Xaa₃₉₄ is Leu or Pro
 Xaa₄₀₂ is Arg or Lys
 Xaa₄₀₄ is Ser or Pro
 Xaa₄₁₃ is Ser or Phe
 Xaa₄₂₂ is Glu or Gly
 Xaa₄₂₈ is Gly or Arg
 Xaa₄₂₉ is Pro or Leu
 Xaa₄₃₅ is Gln or Arg
 Xaa₄₄₇ is Arg or Gly
 Xaa₄₅₃ is Asn, Ser, or Ile
 Xaa₄₅₉ is Met or Thr, and
 Xaa₄₈₅ is Asp or Gly.

3. An isolated nucleic acid sequence encoding a polypeptide with isoflavone synthase activity.

4. An isolated nucleic acid sequence encoding a polypeptide with isoflavone synthase activity wherein the nucleic acid sequence is not the nucleic acid sequence set forth in SEQ ID NO:9.

5. The isolated nucleic acid sequence of Claim 1 at least 85% identical to the nucleic acid set forth in SEQ ID NO:1.

6. The isolated nucleic acid sequence of Claim 1 at least 90% identical to the nucleic acid set forth in SEQ ID NO:1.

7. The isolated nucleic acid sequence of Claim 1 wherein the nucleic acid hybridizes to the nucleic acid set forth in SEQ ID NO:1

8. The isolated nucleic acid sequence of Claim 1 wherein the encoded polypeptide comprises an amino acid sequence that is at least 95% identical to the amino acid sequence set forth in SEQ ID NO:2.

9. The isolated nucleic acid sequence of Claim 1 selected from the group consisting of SEQ ID NOs:1, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 47, 54, 56, 58, and 60.

10. The isolated nucleic acid sequence of Claim 1 encoding the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NOs:2, 10, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 48, 55, 57, 59, 61, and 66.

11. A chimeric sequence comprising the nucleic acid sequence of Claim 1 operably linked to suitable regulatory sequences.

12. A transformed host cell comprising the chimeric sequence of Claim 11.

13. The transformed host cell of Claim 12 further comprising a second chimeric sequence comprising a nucleic acid sequence encoding a polypeptide that regulates expression of at least one enzyme of the phenylpropanoid pathway.

14. The transformed host cell of Claim 13 wherein the second chimeric sequence comprises a chimera containing the maize R region between the region encoding the C1 DNA binding domain and the C1 activation domain.

15. The transformed host cell of Claim 12 wherein the host cell is a eukaryotic cell.

16. The eukaryotic cell of Claim 13 wherein the cell is a yeast cell.

17. The eukaryotic cell of Claim 15 wherein the cell is a plant cell.

18. The plant cell of Claim 17 wherein the cell is a soybean cell.

19. The plant cell of Claim 17 wherein the cell is a corn cell.

20. A plant comprising in its genome the chimeric sequence of Claim 11.

21. The plant of Claim 20 further comprising in its genome a second chimeric sequence comprising a nucleic acid sequence encoding a polypeptide that regulates expression of at least one enzyme of the phenylpropanoid pathway.

22. The plant of Claim 20 wherein the plant is a soybean plant.

23. The plant of Claim 20 wherein the plant is a corn plant.

24. A seed from the plant of Claim 20.

25. A seed from the plant of Claim 21.

26. A method of altering the level of expression of isoflavone synthase in a host cell comprising:

- (a) transforming a host cell with the chimeric sequence of Claim 11;
- (b) optionally transforming the host cell with a second chimeric sequence comprising a nucleic acid sequence encoding a polypeptide that regulates expression of at least one enzyme of the phenylpropanoid pathway; and
- (c) growing the transformed host cell produced in step (a) or step (b) under conditions that are suitable for expression of the chimeric sequence

wherein expression of the chimeric sequences result in production of altered levels of isoflavone synthase in the transformed host cell.

27. A method of increasing the amount of an isoflavonoid in a host cell comprising:

- (a) transforming a host cell with the chimeric sequence of Claim 11;
- (b) optionally transforming the host cell with a second chimeric sequence comprising a nucleic acid sequence encoding a polypeptide that regulates expression of at least one enzyme of the phenylpropanoid pathway; and
- (c) growing the transformed host cell produced in step (a) or step (b) under conditions that are suitable for expression of the chimeric sequence

wherein expression of the chimeric sequences results in production of an amount of an isoflavonoid in the transformed host cell that is greater than the amount of the isoflavonoid that is produced in a cell that is not transformed with the chimeric sequence of Claim 11.

28. The method of Claim 26 wherein the isoflavonoid is selected from the group consisting of genestein and daidzein.

29. The method of Claim 26 or Claim 27 wherein the host cell is a eukaryotic cell.

30. The method of Claim 26 or Claim 27 wherein the eukaryotic cell is a yeast cell.

31. The method of Claim 26 or Claim 27 wherein the eukaryotic cell is a plant cell.

32. The method of Claim 31 wherein the plant cell is a soybean cell.

33. The method of Claim 31 wherein the plant cell is a corn cell.

34. A method of producing a plant with increased isoflavonoid content comprising

- (a) transforming a plant cell with the chimeric sequence of Claim 11;
- (b) optionally transforming the plant cell with a second chimeric sequence comprising a nucleic acid sequence encoding a polypeptide that regulates expression of at least one enzyme of the phenylpropanoid pathway; and
- (c) growing the transformed plant cell under conditions that promote the regeneration of a whole plant from the transformed cell

wherein the transformed plant regenerated from the transformed cell produces an amount of an isoflavonoid that is greater than the amount of the isoflavonoid that is produced in a plant that is regenerated from a plant cell that is not transformed with the chimeric sequence of Claim 11.

35. The method of Claim 34 wherein the plant is a soybean plant.

36. The method of Claim 34 wherein the plant is a corn plant.

37. The transgenic plant produced by the method of Claim 34.

38. The transgenic plant of Claim 37 wherein the plant is a soybean plant.

39. The transgenic plant of Claim 37 wherein the plant is a corn plant.

40. A seed from the plant of Claim 37.

41. A method of obtaining a nucleic acid sequence encoding all or a substantial portion of the amino acid sequence encoding a plant isoflavone synthase comprising

- (a) probing a cDNA or genomic library with the nucleic acid sequence of Claim 1;
- (b) identifying a DNA clone that hybridizes with the nucleic acid sequence of Claim 1;
- (c) isolating the DNA clone identified in step (b);
- (d) sequencing the cDNA or genomic sequence that comprises the clone isolated in step (c); and
- (e) demonstrating the functional expression of isoflavone synthase mediated by the cDNA or genomic sequence sequenced in step (d)

wherein the sequenced nucleic acid sequence encodes all or a substantial portion of the amino acid sequence encoding a plant isoflavone biosynthetic enzyme.

42. A method of obtaining a nucleic acid sequence encoding all or a substantial portion of an amino acid sequence encoding a plant isoflavone synthase comprising:

- (a) synthesizing an oligonucleotide primer corresponding to a portion of the sequence set forth in a member of selected from the group consisting of SEQ ID NOs:1, 9, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 47, 54, 56, 58, and 60;
- (b) amplifying a cDNA insert present in a cloning vector using the oligonucleotide primer of step (a) and a primer representing sequences of the cloning vector to produce an amplified nucleic acid sequence; and
- (c) demonstrating the functional expression of isoflavone synthase mediated by the amplified nucleic acid sequence produced in step (b)

wherein the amplified nucleic acid sequence encodes all or a substantial portion of an amino acid sequence encoding a plant isoflavone synthase.

43. The method of Claim 42 wherein the oligonucleotide primer is selected from the group consisting of SEQ ID NOs:5, 6, 7, 8, 11, 12, 13, 14, 41, 42, 49, 50, and 51.

44. The product of the method of Claim 41.

45. The product of the method of Claim 42.

46. A method of altering the level of isoflavonoids in a cell of Claim 12 comprising exposing said cell to a phenylpropanoid pathway altering agent.

47. The method of Claim 46 wherein said agent is selected from the group consisting of a transcription factor and stress.

48. The method of Claim 47 wherein stress is selected from the group consisting of ultraviolet light, temperature, pressure, and phosphate level.

49. The method of Claim 47 wherein said transcription factor is a maize C1 myb-type transcription factor and a myc-type transcription factor R

50. The method of Claim 47 wherein said transcription factor is a chimera containing the maize R region between the C1 DNA binding domain and the C1 activation domain.